Air Cleanliness Test on CO_2 Incubator

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Introduction

Incubators are used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature, humidity, and other conditions such as the carbon dioxide (CO_2) content of the atmosphere inside. Incubators are essential for a lot of experimental work in cell biology, microbiology, and molecular biology.

The simplest incubators are insulated boxes with an adjustable heater, typically going up to 60 to 65 °C (140 to 150 °F). More elaborate incubators can also include the ability to control humidity or CO_2 levels. This is important in the cultivation of mammalian cells, where the relative humidity is typically 85%-90% and a slightly acidic pH is achieved by maintaining a CO_2 level of 5%.

One of the most critical aspects of an aseptic manufacturing facility is the amount of viable microorganisms and non-viable particulates within a controlled area. Good environment control to ensure contaminant-free chamber is also important to support cell growth. To control the chamber air cleanliness, Esco CO_2 incubators are equipped with a SteriSafe™ ULPA filtration system.

The SteriSafe™ system utilizes a fan to re-circulate air inside the incubator through an ULPA filter to capture the airborne contaminants. The objective of this system is to achieve a biosafety cabinet comparable cleanliness level of at least ISO Class 5 inside the incubator chamber, which was investigated in this study.

Procedure

A MetONE Model A2400-LLD-220 discrete laser particle counter having the particle size detection threshold of 0.5µm was used to study the air cleanliness level on Esco CCL-170 CO_2 Incubator. The number of particles with a size between 0.5 to 5 micron was measured, as per Guidelines for Environmental Monitoring.

The particle count nozzle was placed at the center of the chamber, with the tube going out through the door seal and connected to the particle counter. The tests were performed under the following conditions:

1. With ULPA filter installed
2. With ULPA filter not installed

The incubator door was opened for 15 minutes to simulate a worst case condition, where the number of particles inside the chamber is in equilibrium with the number of particles in the room, which is a BSL-2 microbiology lab, without a room air filtration system.

Below is the table indicating the ISO Cleanliness with the corresponding number of particles of different sizes, per one cubic meter of volume:

	Maximum particles / m^3					
Class	≥ 0.1 µm	≥ 0.2 µm	≥ 0.3 µm	≥ 0.5 µm	≥ 1 µm	≥ 5 µm
ISO 1	10	2	0	0	0	0
ISO 2	100	24	10	4	0	0
ISO 3	1,000	237	102	35	8	0
ISO 4	10,000	2,370	1,020	352	83	0
ISO 5	100,000	23,700	10,200	3,520	832	29
ISO 6	1,000,000	237,000	102,000	35,200	8,320	293
ISO 7	10,000,000	2,370,000	1,020,000	352,000	83,200	2,930
ISO 8	100,000,000	23,700,000	10,200,000	3,520,000	832,000	29,300
ISO 9	1,000,000,000	237,000,000	102,000,000	35,200,000	8,320,000	293,000

Because the MetOne particle counter used for this experiment takes air sample of 1 cubic foot per minute, below is the ISO Cleanliness with the corresponding number of particles of different sizes, per one cubic foot of volume:

	Maximum particles / ft^3					
Class	≥ 0.1 µm	≥ 0.2 µm	≥ 0.3 µm	≥ 0.5 µm	≥ 1 µm	≥ 5 µm
ISO 1	0	0	0	0	0	0
ISO 2	3	1	0	0	0	0
ISO 3	28	7	3	1	0	0
ISO 4	283	67	29	10	2	0
ISO 5	2,833	671	289	100	24	1
ISO 6	28,329	6,714	2,890	997	236	8
ISO 7	283,286	67,139	28,895	9,972	2,357	83
ISO 8	2,832,861	671,388	288,952	99,717	23,569	830
ISO 9	28,328,612	6,713,881	2,889,518	997,167	235,694	8,300

Results

Door Closed	1. With SteriSafe™ ULPA filtration system, number of particles / ft³			
(Minutes)	0.5 µm	1 µm	3 µm	5 µm
1	4577	124	6	0
2	3897	12	0	0
3	2600	8	0	0
4	1259	2	0	0
5	623	1	0	0
6	304	0	0	0
7	267	0	0	0
8	212	0	0	0
9	167	0	0	0
10	107	0	0	0
11	80	0	0	0
12	56	0	0	0
13	11	0	0	0

Door Closed	2. Without ULPA filtration system, number of particles / ft³			
(Minutes)	0.5 µm	1 µm	3 µm	5 µm
1	4720	1385	4	0
2	4216	1678	3	0
3	3502	1300	0	0
4	3151	1336	1	0
5	3107	1213	0	0
6	2924	1119	0	0
7	2326	924	0	0
8	1808	156	0	0
9	1800	152	0	0
10	1461	78	0	0
11	1448	66	0	0
12	1418	54	0	0
13	1320	48	0	0

Observation and Conclusion

1. The starting point / initial number of particles is approximately the same between the test with and without SteriSafe™ ULPA filtration system.

2. The incubator with SteriSafe™ system managed to reach ISO Class 5 cleanliness at 11 minutes after the door was closed. This is the minimum cleanliness requirement for biological safety cabinets.

3. With the SteriSafe™ system, the cleanliness level further improves to near ISO Class 4 at 13 minutes after the door was closed, suggesting that if we wait further, we can even reach ISO Class 4, which is comparable to respectable biological safety cabinets.

4. Without the SteriSafe™ system, the incubator was still far from reaching ISO Class 5 even at 13 minutes after the door was closed. Although approaching ISO Class 6, the cleanliness level was still at ISO Class 7, which is the same classification as when the door is just closed.

5. Without the SteriSafe™ system, the cleanliness level improvement seems to approach steady state condition at 13 minutes after the door was closed. Despite further study is required to find the steady state point, it seems unlikely for the incubator to reach ISO Class

References

[1] Guidelines on Test Methods for Environmental Monitoring for Aseptic Dispensing Facilities. A Working group of the Scottish Quality Assurance Specialist Interest Group. Third Edition. November 2002

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[3] Lincoln CK, Gabridge MG. Cell culture contamination: sources, consequences, prevention and elimination. In: Mather JP, Barnes D, ed. Animal cell culture methods. San Diego: Ac0ademic Press, 1998:49–65.

[4] United States Pharmacopeia and National Formulary USP30 <1116> “Microbiological Evaluation of Cleanrooms and Controlled Environments”